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Therapeutic targets in myeloma bone disease

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Running title: Targeting myeloma bone disease

Abbreviation: BM, bone marrow; BMSCs, bone marrow stromal cells; CCR1, C-C chemokine receptor type 1; Dkk-1 dickkopf-1; ICAM-1, intercellular adhesion molecule 1; IGF-1, insulin-like growth factor 1; IL, interleukin; IMiDs, immunomodulatory drugs; G-CSF, granulocyte colony stimulating factor; GM-CSF, granulocyte-macrophage colony-stimulating factor; Gfi-1, transcriptional repressor growth factor independent 1; M-CSF, macrophage colony-stimulating factor; MGUS, monoclonal gammopathy of undetermined significance; MIP-1α, macrophages inflammatory protein-1α; miRNAs, micro RNAs; MM,

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multiple myeloma: MBD, MM bone disease; MMP, matrix metalloproteinase; NK, natural killer cells; OAF, osteoclast-activating factors; OB, osteoblast; OC, osteoclast; OCY, osteocyte: RANKL, receptor activator of nuclear factor-κB ligand; SDF-1, stromal-derived growth factor-1; TGF-β, Transforming growth factor β; TNF-α, tumor necrosis factor α; VCAM, vascular cell adhesion molecule; VEGF, vascular endothelial growth factors;

Abstract

Multiple myeloma (MM) is the second-most-common hematologic malignancy and is characterized by a clonal proliferation of neoplastic plasma cells within the bone marrow. MM is the most frequent cancer involving the skeleton, causing osteolytic lesions, bone pain, and pathological fractures that dramatically decrease MM patients' quality of life and survival. MM bone disease (MBD) results from uncoupling of bone remodelling in which excessive bone resorption is not compensated by new bone formation, due to a persistent suppression of osteoblast activity. Current management of MBD includes anti-resorptive agents i.e. bisphosphonates and denosumab that are only partially effective due to their inability to repair the existing lesions. Thus, research into agents that prevent bone destruction and more importantly repair existing lesions by inducing new bone formation, is of the utmost importance. This review discusses the mechanisms regulating the uncoupled bone remodelling in MM, and summarizes current advances in the treatment of MBD.

Bullet point summary -

'What is already known',
1. MM is still incurable and 80% of patients develop lytic bone disease.
2. The majority of lytic lesions do not repair even when the patients are in complete remission.

'What this study adds'
1. We review and summarize recent advances in the pathogenesis of MBD.
2. Provide possible new mechanism(s) that can be targeted to build bone.

'Clinical significance'.
1. Current MBD treatments are only anti-resorptive and not anabolic.
2. Provides support for potential safe anabolic agents that actively stimulate bone formation.

1. Introduction

Multiple myeloma (MM) is the second most common hematological malignancy that results from an uncontrolled clonal proliferation of malignant plasma cells, in the bone marrow microenvironment. It is characterized by the production and accumulation of paraproteins (monoclonal immunoglobulins) detectable in patient's blood and urine. Approximately 32,110 new cases will be diagnosed in the United States in 2019 with 12,960 deaths due to MM (Siegel, Miller & Jemal, 2019). The incidence of MM is 2-3 times higher in African Americans than in Caucasians. The overall 5-year survival rate is 50%. Clinically, MM is a debilitating disease characterized by bone destruction (> 80% of patients), hypercalcemia (30% of patients), acute renal failure (20% of patients), anemia (60 % of patients) and reductions in normal gamma globulins (immunoparesis).

Despite the fact that survival rates have greatly improved in recent years, MM is incurable in the majority of patients since more than 80% of patients develop bone diseases (MBD) over the course of their disease (Roodman, 2010). MBD represents a major issue in the current management of MM. Skeletal involvement is responsible for some of the most devastating complications associated with MM, including pathological fractures that can occur in 50-60% of patients, causing debilitating pain and spinal cord compression syndromes which reduce quality of life and increase mortality risk by up to 20% (Saad, Lipton, Cook, Chen, Smith & Coleman, 2007).

MBD is caused by interactions between myeloma and cells of the bone microenvironment, that result in the formation of lytic bone lesions and generalized bone loss. These interactions lead to a profound deregulation of the normal bone remodeling process with excessive osteoclastic bone resorption accompanied by a substantial suppression of osteoblastic bone formation, thus results in little or no new bone deposition (Roodman, 2009). Moreover, the majority of lytic lesions do not repair and persist even when the patients are in complete remission and with no evidence of marrow infiltration by MM cells, due to the severe and persistent suppression of osteoblast (OB) activity that characterizes MM (Roodman, 2011) (Fig1).

Current treatments for MBD such as bisphosphonates and denosumab are only anti-resorptive and reduce further bone destruction by osteoclasts but do not actively stimulate bone formation. Recently, several bone anabolic agents have been tested in preclinical models of MBD and studies to develop novel safe anabolic agents for MBD are ongoing. This review discusses current and novel therapeutic approaches that target MBD.

2. Pathophysiology of Myeloma Bone Disease

The normal physiologic bone remodeling that regulates the balance between osteoclast (OC) and OB activity is uncoupled in MBD. The bone marrow microenvironment consists of a cellular compartment and a mineralized extracellular matrix. Marrow cells and components of the marrow microenvironment including OCs, OBs, osteocytes (OCYs), immune cells and stromal cells, produce membrane-bound and soluble growth factors, that stimulate the homing, growth, proliferation and drug-resistance of MM cells (Andrews, Kabrah, May, Donaldson & Morse, 2013). MM in turn stimulate OC formation directly or by physically interacting with stromal cells and OBs, to produce factors that drive OC formation and suppress OB activity, thereby creating a "vicious cycle" that increases bone resorption and tumor burden (Marino & Roodman, 2017). Conversely, OB function is extremely suppressed or absent, resulting in purely lytic bone lesions that rarely repair. Immobilized matrix-derived growth factors, such as transforming growth factor β (TGF-β), are released from the resorbed bone matrix, further promoting MM cell growth.

The physiological state of the microenvironment is a key element in the tumor progression as it can be both tumor-promoting or tumor-restricting. The highly vascularized metaphysis of the bone is the preferential site of MM engraftment and the perivascular niche has been shown to regulate MM cell homing and dissemination (Ribatti, Basile, Ruggieri & Vacca, 2014). The uncontrolled growth of disorganized new MM-associated vessels supports MM cell growth and chemoresistance (Moschetta et al., 2016). Finally, the hypoxic microenvironment further activates vasculogenesis (Storti et al., 2013) and enhance the genomic instability of MM cells, selecting for dormant MM cell clones thus contributing to MM drug resistance (Hu, Van Valckenborgh, Menu, De Bruyne & Vanderkerken, 2012)

The tumor-immune interactions in MM are distinct since MM cells are themselves immune cells (plasma cells) and localise primarily in the bone marrow (BM), a specialized tissue for survival of immune cells. Here they interact with bone cells, suggesting a potential role for these interactions in regulating both tumor progression and tumor immunity. The immunosuppressive environment that is associated with MM, contributes to the immune escape of the MM cells because of their inadequate antigen presentation (Hideshima, Mitsiades, Tonon, Richardson & Anderson, 2007), indicating that enhancing immune surveillance may play a role in the prevention of MM disease progression.

Furthermore, recent studies showed interplay between MM cells and marrow adipocytes, actively support the proliferation and migration of MM cells, protect MM cells from chemotherapy-induced apoptosis and disrupt haematopoiesis, leading to the disruption of

immune function and contribute to myeloma-induced bone disease (Trotter, Gibson, Sherpa, Gowda, Peker & Yang, 2016) (Liu et al., 2019) (Fig 2).

3. Cellular components of myeloma bone disease

Bone marrow stromal cells

Bone marrow stromal cells (BMSCs) are a special class of multi-potent cells that represent a common progenitor for OBs, OCYs, adipocytes, chondrocytes, muscle cells and most of the stromal cells in the bone marrow (Lindner, Kramer, Rohwedel & Schlenke, 2010). BMSCs function is highly influenced by the surrounding microenvironment, and thus, it is altered in MM and contributes to multiple stages of the pathophysiology of MBD (Olechnowicz & Edwards, 2014). The capability of MM-BMSCs to differentiate into functional OBs is severely compromised although is still unclear whether these modifications are permanent or require the presence of MM cells (Kassen et al., 2014). MM-BMSCs express a distinct genomic profile when compared to healthy-BMSCs (Munshi & Avet-Loiseau, 2011). BMSCs isolated from MM patients secrete high level of tumor promoting and anti-apoptotic factors, such as interleukin (IL) IL-6 (Harding et al., 2018), insulin-like growth factor 1 (IGF-1), vascular endothelial growth factors (VEGF), tumor necrosis factor α (TNF-α), stromal-derived growth factor-1α (SDF-1) and express higher level of adhesive molecules intercellular adhesion molecule 1 (ICAM-1) and vascular cell adhesion molecule 1 (VCAM-1), which contribute to drug resistance in MM. Moreover, adhesive interactions between MM cells and BMSCs are responsible for the production of BMSCs-derived receptor activator of nuclear factor-κB ligand (RANKL), macrophage colony-stimulating factor (M-CSF), IL-11 and IL-6 and MM-derived macrophages inflammatory protein-1α (MIP-1α), IL-3 and VEGF that regulate osteoclastogenesis (reviewed in (Marino & Roodman, 2017)). In addition, MM-BMSCs also produce exosomes containing proteins, DNA and microRNAs that can prime the bone microenvironment to promote MM dissemination and support MM growth (Guo et al., 2017).

Over the past years there has been great interest in understanding epigenetic and transcriptional signatures changes that MM cells induce on BMSCs (reviewed in (Adamik, Galson & Roodman, 2018). We recently showed that epigenetic-based mechanisms are involved, at least in part, in the persistence osteogenic suppression of MM-BMSCs (Adamik et al., 2017; D'Souza et al., 2011). We found that upregulation and enhanced binding of the Runx2-transcriptional repressor growth factor independent 1 (Gfi-1) to the Gfi-1-response element within the Runx2 promoter mediated the sustained suppression of osteogenesis. In

MM-exposed BMSC cells, Gfi-1 acted as a platform for the formation of a repressive complex containing enhancer of zeste homolog 2 (EZH2), histone deacetylase 1 (HDAC1) and lysine-specific demethylase 1 (LSD1). Moreover, Gfi-1 can regulates adipogenesis, suggesting that Gfi-1 may both suppress and shift the differentiation of MM-exposed BMSCs towards adipogenesis (Wang et al., 2016). We showed that blockade of the p62-ZZ-domain of p62, a signalling platform that mediates the inflammatory response in BMSC exposed to MM cells, with a novel small molecule inhibitor XRK3F2, resulted in inhibition of Gfi-1 activity and rescued osteogenesis of MM-exposed BMSCs (Adamik et al., 2018). XRK3F2 prevented deacetylation of the Runx2 promoter and alleviated MM-suppressed osteogenesis *in vitro* (Adamik et al., 2018) as well as *in vivo* (Teramachi et al., 2016). These results suggest that targeting p62 may allow restoration of OB function in patients with MBD. The role of pharmacological treatments that target epigenetic mechanisms suppressing OB differentiation of MM-BMSCs are under investigation (previously reviewed by (Garcia-Gomez, Sanchez-Guijo, Del Canizo, San Miguel & Garayoa, 2014).

Osteoblasts

MBD is characterized by a severe suppression of OBs activity, which results in generalized bone loss due to the inability of the OBs to repair the lesions caused by the excessive osteoclastic resorption (Roodman, 2011). Both soluble factors and physical interaction between MM cells and OB progenitors inhibit OB differentiation and increase apoptosis in OBs (Giuliani & Rizzoli, 2007).

As noted above, we and others showed that the suppression of the activity and function of Runx2 expressed in both mesenchymal and osteoprogenitor cells by MM cells, is principally mediated by direct cell-cell interaction, although soluble factors released by MM cells and cells in the bone microenvironment also contribute to Runx2 suppression (D'Souza et al., 2011; Giuliani et al., 2005). Further, inhibition of Runx2 reduces osteoprotegerin (OPG) (Nelson et al.) secretion resulting in increased osteoclastogenesis. Soluble factors including TGF-β, released by the bone matrix after OC resorption, inhibits OB differentiation (Matsumoto & Abe, 2011). IL-7 also suppresses OB differentiation, prevents bone formation and induces bone loss in animal model of MM. Neutralizing antibodies directed against IL-7, partially reduce MM-down-regulation of Runx2 transcriptional activity and OB suppression (Giuliani et al., 2005).

MM cells also induce high level of TNF-α in the MM-bone marrow microenvironment. TNF-α is known to play a dual role as it increases osteoclastogenesis and inhibits OB differentiation by decreasing Runx2, Osterix, type-1 collagen, osteocalcin, and matrix

deposition and induces apoptosis of mature OBs (Ghali, Chauveau, Hardouin, Broux & Devedjian, 2010). Recently, we showed that IL-7 and TNF-α also induce Gfi-1, potentiating OB suppression (D'Souza et al., 2011).

IL-3, has also been shown to play a dual role in MBD. It significantly increases OC activity, prevents OB differentiation and inhibits mineralization of mature OBs via inducing Activin A, a TGF-β cytokine member (Vallet et al., 2010).

The Wnt signalling pathway is an essential regulator of OB proliferation, differentiation and survival. Dkk-1 is an extracellular antagonist of the Wnt pathway that prevents the binding of Wnt to the low-density lipoprotein receptor-related protein (LRP) 5/6, downregulating Runx2 activity and OB differentiation (Baron & Kneissel, 2013). Dickkopf-1 (Dkk-1) protein levels, produced by both MM and MM-BMSCs, are increased in MM patients with skeletal lesions (Fowler, Mundy, Lwin & Edwards, 2012; Kaiser et al., 2008). Dkk-1 also promotes osteoclastogenesis and bone resorption by modulating RANKL and OPG expression in OBs (Spencer, Utting, Etheridge, Arnett & Genever, 2006).

Osteocytes

OCYs represent ~95% of bone cells and regulate the response of bone to mechanical stress. OCYs contribute to MBD by regulating both OB and OC activity through the secretion of factors including sclerostin, an inhibitor of the canonical Wnt/β catenin signalling (Delgado-Calle, Sato & Bellido, 2017), and RANKL (Delgado-Calle, Bellido & Roodman, 2014). Serum levels of sclerostin correlate with advanced MBD and poor patient survival (Terpos et al., 2012a). Recently, we and others reported that administration of neutralizing monoclonal anti-sclerostin antibodies, in preclinical model of MM, increases bone mass and reduces osteolytic lesions (Delgado-Calle et al., 2017; McDonald et al., 2017) and in combination with conventional anti-MM therapy such as carfilzomib, significantly reduces tumor burden (Eda et al., 2016). Physical interactions between MM cells and OCYs up-regulates Sost/sclerostin expression in OCYs and stimulate MM cell proliferation via a Notch-mediated pathway (Delgado-Calle et al., 2016), further contributing to the profound OB suppression.

Osteoclasts

MM cells directly stimulate OC formation by releasing pro-inflammatory osteoclast-activating factors (OAF), including RANKL, MIP-1α, TNF-α, IL-3, IL-6, parathyroid hormone related protein (PTHrP) and hepatocyte growth factor (HGF). As a result of osteoclastic bone resorption, immobilized growth factors are released from the matrix, such

as IGF-1, FGF and TGF-β and promote MM cell growth, exacerbating the vicious cycle of MBD (reviewed in (Marino & Roodman, 2017)). Moreover, we and others recently reported that OCs contribute to the increased MM-microvessel density via the production of MM-derived VEGF and OC-derived osteopontin and MMP-9 contributing to disease progression (Giuliani, Storti, Bolzoni, Palma & Bonomini, 2011). RANKL is the major regulator of both normal and pathological bone remodeling is mainly expressed and released by stromal cells, OBs and OCYs and is also secreted by activated T lymphocytes (Wada, Nakashima, Hiroshi & Penninger, 2006). RANKL binds its receptor RANK, a member of the TNF receptor superfamily, expressed on the surface of OC precursors and mature OC, stimulating OC differentiation, survival and activity. OPG is the soluble decoy receptor for RANKL, produced by BMSCs and OCYs and blocks the interaction between RANKL and its receptor on OCs (Boyce & Xing, 2008). An increased RANKL/OPG ratio in MM patients correlated with poor prognosis and reduced survival (Terpos et al., 2003). It is unclear however if MM cells directly produce RANKL or only induce its production by acting on cells in the bone microenvironment (Giuliani, Colla & Rizzoli, 2004). In addition, OCY apoptosis induced by MM cells has recently been shown to contribute to MBD due to the release of osteocytic RANKL (Giuliani et al., 2012). Studies in preclinical models of MBD demonstrated that blocking RANKL, via administration of recombinant OPG or RANK-Fc, significantly decreased osteolytic lesions and tumor growth in mice.

MIP-1α is a chemokine mainly produced by MM cells that enhances homing of MM cells to the marrow as well as their proliferation and adhesion to BMSCs cell. MIP-1α acts as a chemotactic factor for monocytes and OC precursors directly inducing OC formation by binding to its receptor C-C chemokine receptor type 1 (CCR1) (Tsubaki et al., 2010) or by enhancing the activity of RANKL and IL-6 (Oyajobi et al., 2003). Recently, MIP-1α was shown to directly impair OB mineralization via downregulation of osteocalcin expression and osterix modulation. Preclinical studies using the murine 5TGM1 model of MBD, showed that blocking CCR1 with the small molecule CCR1 antagonist, MLN3897 decreases tumor burden and bone destruction (Vallet et al., 2007) and partially reverses the inhibitory effects of MIP-1α on OBs (Vallet et al., 2011). Small molecule CCR1 inhibitors are currently under development.

TNF-α produced by MM cells, MM-activated T cells and MM-BMSCs, enhances OC differentiation by stimulating a number of pro-survival signaling pathways including NF-κB, MAP-kinases and PI3K/Akt pathways and enhances the effects of RANKL (Boyce et al., 2005). Moreover, TNF-α induces expression of pro-survival genes in MM cells, promotes

growth and confer chemotherapeutic resistance to apoptotic stimuli by activating several pathways, including both canonical and non-canonical NF-κB pathway (Li, Chen, Campbell, Bonavida & Berenson, 2008). Furthermore, TNF-α increases the expression of VCAM1 and the secretion of RANKL and IL-6 by stromal cells (Teramachi et al., 2016), enhancing stromal cell support of osteoclastogenesis and MM cell growth and positively correlates with increased angiogenesis in MM patients (Bolkun et al., 2014).

IL-3 also affects osteoclastic bone resorption by cooperating with RANKL and MIP-1α (Lee et al., 2004). IL-3 is produced by both MM cells and T cells in MM patients and its levels are elevated in the plasma of approximately 70% of MM patients. We reported that the mechanism of action of IL-3 in OCs, also involves Activin A produced by macrophages, and showed that activin A mediates the effects of IL-3 on osteoclastogenesis *in vivo* (Silbermann et al., 2014).

PTHrP is a secreted factor, functionally analogous to the parathyroid hormone (PTH), that controls a number of developmental, physiological and pathological processes (Rosen, 2013) and it is a major regulator of calcium homeostasis . PTHrP not only plays a key role in osteolytic metastases by solid tumors, but also enhances MM cell survival and reinforces MM-production of osteoclastogenic factors such as RANKL and monocyte chemoattractant protein-1 (MCP-1) (Cafforio et al., 2014). PTHrP induces the expression of the osteoblastic transcriptional repressor gene E4BP4, exacerbating MBD (Silvestris, Cafforio, De Matteo, Calvani, Frassanito & Dammacco, 2008).

Semaphorin 4d (Sema4D) also plays a role in MBD. It was recently reported that OCYs are the major source of Sema4D (Suvannasankha et al., 2016) along with MM cells and OCs (Terpos et al., 2012b). Sema4D increases OCs activity and suppresses of OBs differentiation and motility (Terpos et al., 2012b). A Sema4D antibody is currently in clinical trial for breast cancer bone metastasis (Patnaik et al., 2016).

Adipocytes

Bone marrow has a significant fat content and marrow adipocytes interact with MM cells, contributing to MBD. The C/EBPα transcription factor and the master adipocyte regulator peroxisome proliferator–activated receptor γ (PPARγ) regulate the differentiation of MSCs into adipocytes. The dynamic relationship and reciprocal repression between levels of marrow adipocytes and OBs is complex and is likely to play a functional role in both skeletal metastasis and MBD (Chen et al., 2016). Adipocytes serve as lipid reserves and may promote

tumor growth and MBD by secreting specific endocrine signalling molecules, including adiponectin and growth factors (leptin, TNFα, MCP-1, and insulin) (reviewed in (McDonald, Fairfield, Falank & Reagan, 2017)). Although a large prospective trial found no association between leptin levels and MM risk, instead, levels of adiponectin have been found to inversely correlate with MM pathogenesis suggesting that it may represent a novel therapeutic target in MBD (Fowler et al., 2011). Recently, Liu et al. demonstrated that MM cells reprogram adipocytes via increasing PPARγ' methylation and altering adipokine production. These changes results in an enhanced osteoclastogenesis and suppression of osteoblastogenesis (Liu et al., 2019).

Immune cells

The immune cells that infiltrate tumors belong to adaptive immunity, characterized by antigen specificity and immunologic memory and innate immunity, typically lacking immunologic memory, with the exception of some subsets of natural killer (NK) cells. The tumor-immune interactions in MM represent a balance of pro- and anti-tumor interactions. All these types of immune cells have subsets that can mediate both pro- and antitumor effects and the balance between the two mediates an effective immune response in MM.

T cells

T cells arise from lymphoid stem cells and after maturation are characterized by the production and expression of antigen-specific, MHC-restricted receptor complexes (TCR/CD3) or subcomponents.

CD4/CD8 T-Cells: Cytotoxic CD8+ T cells (Th1/effector) mediate tumor protection, while regulatory CD4+ T cells (Th2/helper) can promote tumor growth (Dhodapkar, Borrello, Cohen & Stadtmauer, 2017). In patients with monoclonal gammopathy of undetermined significance (MGUS), despite a significant tumor burden, the disease does not progress but subsists in a 'plateau phase'. This phenomenon is associated with the presence of expanded T-cell clones that have the immunophenotype of effector memory T cells ($CD3^+CD8^+CD57^+$) and an improved survival (Brown et al., 2009). Identification of pre-malignancy-specific effector T cells in patients with MGUS represents an indirect evidence of host anti-tumor immune activity. Clonally expanded T-cell populations have a much higher proliferative

capacity in MM patients who have long-term survival than in MM patients with shorter survival (Dhodapkar, Krasovsky, Osman & Geller, 2003).

Th17

Th17 cells are an important constituent of the adaptive immune response defined as pro-inflammatory CD4+ T helper cells that secrete the cytokine IL-17 and IL-22. IL-17 induces expression of a number of chemokines and cytokines including IL-6, TGF-β, granulocyte colony stimulating factor (G-CSF) or granulocyte-macrophage colony-stimulating factor (GM-CSF), matrix metalloproteinase (MMP), and ICAM-1 in a variety of cell types, including BMSC. TGFβ and IL-6, both expressed at high levels in MM bone marrow, influence the generation of Th17 cells and consequently modulate antitumor immune responses (Prabhala et al., 2010). A recent study found a significantly higher Th17 and lower Tregs in the long-term survival MM patients when compared with the MM group, indicating that promoting immune responses is therefore an important area of therapeutic intervention in MM (Bryant et al., 2013).

Regulatory T (Tregs) cells

CD4+Tregs cells secrete the inhibitory cytokines IL-10, required for their immune suppressive responses *in vivo* and TGF-β involved in NK cells inhibition and T-cell suppression (Strauss, Bergmann, Szczepanski, Gooding, Johnson & Whiteside, 2007), including those mounted by Th17 cells (Shen, Yuan, Liu & Hu, 2012)

CD8+ Tregs have been identified as well but their mechanism of action involves TNFα and CCL4 instead of TGF-β or IL-10, causing cells to become cytostatic (Muthu Raja et al., 2012).

and express high levels of cytotoxic T lymphocyte-associated antigen 4 (CTLA-4) and GITR. Tregs play an important role in the maintenance of self-tolerance and the modulation of overall immune responses against infections and tumor cells. In MM, abnormal Tregs may contribute to the myeloma-related immune dysfunction (Atanackovic et al., 2008).

Natural Killer (NK) cells

NK cells are lymphocytes defined as CD56+CD3- that function as cytotoxic cells in a non-HLA-restricted manner. Phenotypic studies showed that, NK cells from MM patients express programmed cell death protein 1 (PD-1), compared to healthy individuals, indicating that MM cells induce functional changes to the immune system in order to create an

immunosuppressive environment favourable for its growth (Benson et al., 2010). NK cells also express a variety of surface receptors, including NKG2D that is critical for the recognition and lysis of MM cells (Talebian et al., 2014). Plasma cells, including MM cells, express MHC class I polypeptide-related sequence A (MICA) antigen that can shed and circulate as a soluble form (sMICA) in the plasma. MICA suppresses NK cells by binding to NKG2D. The MICA expression levels on plasma cells, as well as sMICA levels correlate with MM progression. MGUS patients express high levels of MICA on their plasma cells compared to normal donors and have high levels of anti-MICA antibodies, whereas MM patients express intermediate MICA levels but high sMICA levels and do not have anti-MICA antibodies (Jinushi et al., 2008).

4. Therapeutic targeting of multiple myeloma bone disease

Novel treatments have improved patient's quality of life and overall survival although MM remains incurable. Bone-targeted therapy has become primarily important has the life expectancy of MM patients has significantly increased. MBD treatments are complex and aim mainly to reduce the development of new osteolytic lesion and prevent the onset of bone pain, spinal cord compression, pathological fracture and hyperalcemia. Moreover, recent studies have shown that restoring normal bone remodelling may synergise with conventional anti-MM therapies in reducing tumour proliferation and further preventing the development of new bone disease. Current MBD management utilizes a combination of chemotherapy, localized radiotherapy and surgery.

4.1 Current Therapies

Antiresorptive

Bisphosphonates

The standard care for the management of MBD are antiresorptive therapies, such us bisphosphonates (BPs) (Pozzi & Raje, 2011). BPs are pyrophosphate analogues that inhibit farnesyl pyrophosphate synthases, the key regulatory enzyme in the mevalonate pathway. BPs therapies, initially used to prevent calcification of soft tissue, are used for the treatment of excessive bone resorption. BPs selectively binds and are adsorbed to mineral surfaces in bone and internalized by OCs during resorption inducing their apoptosis (Russell & Rogers, 1999).

BPs are classified into two groups with different modes of action and clinical effects: non-nitrogen-containing BPs, such as clodronate and etidronate, are metabolically accumulated as non-hydrolysable analogues of ATP (Frith, Monkkonen, Auriola, Monkkonen & Rogers, 2001). The more potent, nitrogen-containing BPs, such as pamidronate, alendronate and zoledronic acid, are not metabolized and inhibit enzymes of the mevalonate pathway, preventing protein prenylation that cause loss of resorption activity and induction of OC apoptosis (Kavanagh et al., 2006). Oral clodronate, intravenous pamidronate and intravenous zoledronic acid are the commonly used bisphosphonates for treatment of MM-induced skeletal related events, and to reduce new osteolytic lesions, new pathological fractures and hypercalcemia (Anderson et al., 2018). BPs treatment also improves MM-patients' quality of life as it reduces bone pain and spinal cord compression (Gralow & Tripathy, 2007). Results from the MRC Myeloma IX trial proved that zoledronic acid was more effective in reducing skeletal related events and mortality in newly diagnosticated patients with symptomatic MM compared to clodronate (Morgan et al., 2010). This study also demonstrates, as previously showed preclinically, that zoledronic acid reduced tumor burden. Although the mechanism of its anti-tumour effect is still not entirely clear, by inhibiting bone resorption BPS might render the bone environment less favourable to the growth and adhesion to mineralized surfaces of MM cells. Moreover, BPs alone or in combination with various chemotherapeutic agents including doxorubicin, paclitaxel and cyclophosphamide have a pro-apoptotic and anti-proliferative effect on MM cells and enhanced γδ T-cell-mediated immunosurveillance (Modi & Lentzsch, 2012).

The choice of zoledronic acid over pamidronate as first line treatment for MBD is due to its reduced infusion time and side effects although both are equally effective in reducing MBD as indicated by the International Myeloma Working Group (IMWG) (Terpos et al., 2013). In order to define the duration and frequency of intravenous administration of zoledronic acid, the Z-MARK study measured markers of bone turnover, such us urinary N-terminal telopeptide of type I collagen (uNTX) and serum C-terminal telopeptide of type I collagen (CTX), and pyridinoline cross-links (ICTP), concluding that MM-patients should be treated with less frequent dosing of zoledronic acid beyond 1-2 years to reduce the development of skeletal related events (Raje et al., 2016). The major complication associated with the therapy is osteonecrosis of the jaw (which can occur in 3.5% of the patients) that increase with the dose and length of the exposure (Van den Wyngaert, Huizing & Vermorken, 2007). Furthermore, BPs-treated patients can develop acute or chronic renal dysfunction, as kidneys are responsible for 40% of BPs excretion (Pozzi & Raje, 2011) and atypical femoral fractures

due to the reduced bone strength following long-term suppression of bone turnover by antiresorptive agents.

Denosumab

Denosumab (Xgeva®) is a fully human monoclonal antibody that binds with high affinity to the membrane-bound and soluble form of RANKL and prevents its binding to RANK receptor on OC. Denosumab a highly specific as it binds only to RANKL and not to other member of TNF superfamily. Denosumab has been approved for the management of metastatic bone diseases associated with solid tumors (Yee & Raje, 2012) and in patients with osteoporosis - and recently glucocorticoid-induced osteoporosis - for increasing bone density (Lewiecki, 2018). FDA recently approved denosumab for the prevention of skeletal related events in patients with newly diagnosed multiple myeloma as a result of the '482 Study (NCT01345019), an international, Phase III, randomized, double-blind, multicentre clinical trial in which denosumab was compared to zoledronic acid (Raje et al., 2018). This study showed that the overall survival rate was similar in patients treated with zoledronic acid compared to denosumab-treated patients. Denosumab reduced skeletal related events and its efficacy was non-inferior to zoledronic acid with respect to time to the first event. Furthermore, denosumab-treated patients showed significantly lower rates of renal adverse events. Infect, denosumab is not cleared by the kidneys and its use is not restricted in patients with renal insufficiency that represent 25-50% of patients with MM. A single-arm Phase II study of the use of Denosumab in MM patients with renal insufficiency is currently on going. Denosumab could represent an additional standard of care treatment for patients with MBD and would be recommended in patients resistant to bisphosphonate treatment. In postmenopausal women, clinical studies have shown that denosumab discontinuation results in a rebound in the risk of multiple vertebral fracture due to the rapid loss of its positive effects on bone mineral density (Tsourdi et al., 2017). However, specific recommendations on how to discontinue denosumab treatment are needed in MM patients as it has been licensed for continuous use.

Proteasome Antagonists

Proteasome inhibitors, such as bortezomib, and its analogues carfilzomib alone or in combination with steroids and immunomodulatory agents such as dexamethasone and lenalidomide, respectively, have potent anti-MM effects and represent standard of care in MM patients (Kouroukis, Baldassarre, Haynes, Imrie, Reece & Cheung, 2014). Moreover,

bortezomib also directly inhibits OC differentiation and bone resorption, increases osteoblastic bone formation and prevent OCYs apoptosis induced by MM cells (Accardi, Toscani, Bolzoni, Dalla Palma, Aversa & Giuliani, 2015). Although the exact mechanism of action on OB differentiation has not being fully understood, a number of preclinical and clinical studies have suggested that bortezomib increases OB differentiation by upregulating BMP2 production and Runx2 transcriptional activity and reducing OCYs production of sclerostin in patients with relapse/refractory MM (Accardi, Toscani, Bolzoni, Dalla Palma, Aversa & Giuliani, 2015; Terpos et al., 2012a). Furthermore, studies reported that in responsive MM patients treated with bortezomib, serum level of Dkk-1, and marker of bone resorption and RANKL were significantly decreased while level of alkaline phosphates and bone formation markers were increased (Lund et al., 2010).

Immunomodulatory Drugs (IMiDs)

IMiDs such as thalidomide, a glutamic acid derivative, and its analogues lenalidomide, and pomalidomide, are a group of compounds that are often used as both forefront and maintenance therapy for MM (Lee & Borrello, 2016). They target both tumor cells and the microenvironment. IMiDs modulate the host microenvironment mainly by co-stimulation of CD4+ and CD8+ T cells (LeBlanc et al., 2004), augmentation and activation of NK and NKT cells and suppression of Tregs (Quach et al., 2010). The anti-myeloma activity of IMiDs is mediated via and downregulation of adhesion molecules, anti-angiogenesis and anti-inflammatory effects and modulation of cytokine production due to inhibition of TNFα production and activity.

The first generation IMiD, thalidomide, is associated with significant toxicity in older patients. Second generation of IMiD, lenalidomide is a more potent drug with fewer side effects than thalidomide and is commonly used in newly-diagnosed multiple myeloma, relapsed refractory myeloma and as maintenance therapy after autologous stem cell transplantation (ASCT). Pomalidomide, a third generation IMiD, is 10 times more potent than lenalidomide and has shown impressive results in relapsed MM patients and in those refractory to both lenalidomide and bortezomib (Baljevic & Holstein, 2018).

IMiDs and MM-tumor microenvironment interactions.

SDF-1α is a chemokine produced within the BM, which is responsible for the initial homing of plasma cells to the BM by binding their C-X-C chemokine receptor type 4 (CXCR-4, CD184) (Hideshima et al., 2002). It also potentiates the adhesion of MM to BMSC by

modulation of adhesion molecules such as very late antigen (VLA-4) and leukocyte function-associated antigen (LFA-1) on plasma cells and VCAM-1 and ICAM-1 on BMSC. These interactions result in activation of NF-kB, which has a positive feedback loop on the upregulation of intracellular adhesion molecules (Hideshima, Chauhan, Schlossman, Richardson & Anderson, 2001). On the other hand, NF-kB activates transcription of a multitude of cytokines such as VEGF, basic fibroblast growth factors (βFGF), TGF-β, TNFα, IGF-1, IL-10 and IL-6, which all contribute to promote MM cells proliferation (Chauhan et al., 1996). IMiDs inhibit TNFα production by activated macrophages, therefore inhibit the interaction of the MM cells with BMSC by downregulating the adhesion molecules such as leukocyte function-associated antigen (LFA-1) and VLA-4 on MM cells and ICAM-1 and VCAM-1 on BMSC cells (Hideshima, Chauhan, Schlossman, Richardson & Anderson, 2001). By disrupting the direct interaction between MM cells and BMSC, IMiDs reduces the pro-survival effects of IL-6, produced by BMSCs, thus reducing cellular adhesion-mediated drug resistance (Breitkreutz et al., 2008).

IMiDs have anti-angiogenic and anti-inflammatory properties.

Although all IMiDs have anti-angiogenic activity, thalidomide has predominant anti-angiogenic activity whereas lenalidomide and pomalidomide have far superior immune enhancing effects. The anti-angiogenic effects, through inhibition of VEGF, are not due to an anti-proliferative mechanisms directed towards endothelial cells but rather to modulation of the chemotactic factors involved in endothelial cell migration (Dredge et al., 2002). Moreover, IMiDs-induced anti-angiogenesis mechanism involves downregulation of PI3K/Akt signaling pathway (Dredge et al., 2005). IMiDs have broad anti-inflammatory properties inhibiting several pro-inflammatory cytokines which also hold pro-myeloma properties. For example, thalidomide, lenalidomide and pomalidomide inhibit the expression of COX-2 enzyme by shortening the half-life of the mRNA. This results in a net reduction of pro-inflammatory prostaglandins PGE2 production in stimulated PBMCs (Payvandi et al., 2004). PGE2 promotes tumor angiogenesis and IL-6 production (Zhang, Abe, Matsushima, Nishimura, Nawata & Muta, 2005), therefore these drugs exert a potential therapeutic role in MM. The potencies of the three different drugs varies greatly. For example, inhibition of TNFα with lenalidomide was 2000-fold more potent then thalidomide and 20000-fold less potent then with pomalidomide.

IMiDs have anti-osteoclastogenic properties

It has been reported that IMiDs, such as lenalidomide and pomalidomide, inhibited OC development by affecting OC precursors' lineage commitment thalidomide almost

completely abrogated RANKL-induced OC formation by downregulating the expression of PU.1, a major transcriptional factor for osteoclastogenesis (Anderson et al., 2006). Inhibition of bone resorption is associated with a reduction of the expression of cathepsin K and αVβ3-integrin in the OC. A recent study showed that lenalidomide decreased serum bone-remodeling markers in patients with refractory and relapsed MM and blocks OC-derived secretion of growth and survival factors (MIP-1a, BAFF, APRIL) and RANKL secretion from BMSCs, indicating that this agent reduces MM burden and directly prevents osteoclastogenesis (Breitkreutz et al., 2008). IMiDs where not found to stimulate OBs. Bolomsky et. al. indicates that IMiD inhibited OB differentiation in a class-related manner by downregulation of major OB regulators (e.g., Runx2, distal-less homeobox 5, pleiotrophin) and concurrent induction of secreted inhibitors of OB formation (e.g. Dkk1, activin A, gremlin 1). These results emphasis the need for bone anabolic therapeutics in combination with IMDis in myeloma, to counteract the negative impact of prolonged IMiD exposure on bone metabolism (Bolomsky et al., 2014).

4.2 Novel Therapies

Anabolic Therapies

Activin A- Antagonist

Activin A, released by BMSC and OCs, is both an OCs agonist as it synergizes with RANKL and directly induces OC differentiation, as well as an OBs inhibitor (Alves, Eijken, Bezstarosti, Demmers & van Leeuwen, 2013; Silbermann et al., 2014). Results from a Phase I study in postmenopausal women showed that Sotatercept (ACE-011), a soluble activin receptor antagonist, significantly reduced bone resorption marker CTX and tartrate-resistant acid phosphatase (TRACP-5b) and increased alkaline phosphatese, the bone-specific formation marker (Ruckle et al., 2009). Sotatercept is currently in a multicentre Phase II clinical trial in combination with lenalidomide and dexamethasone for patients with relapsed and refractory MM with osteolytic bone lesions (NCT01562405) (Yee et al., 2015). Preliminary data reported that Sotatercept inhibited MM cell growth, improve anaemia and increases bone mass.

Dkk1 Antagonist

Due to its key role in primarily mediate OBs suppression and indirectly enhances OC function in MM (Zhou, Meng, Song & Claret, 2013), Dkk1 represent an attractive therapeutic target for MBD. Results from clinical studies showed a relationship between Dkk1 and MBD

as it is highly produced by MM cells from patients with osteolytic lesions compared to patients with MGUS or healthy donor (Kaiser et al., 2008; McDonald et al., 2017). These studies suggested that Dkk1 can be targeted to stimulate bone formation. Strategies directed towards Dkk1 include neutralizing inhibitors of Wnt signaling, proteasome inhibitors and tumor-produced endothelin-1 (ET-1).

Preclinical studies showed that the anti-Dkk1 antibody BHQ880 increased OB differentiation, upregulated β-catenin levels and downregulated NF-κB activity and reduced IL-6 secretion (Fulciniti et al., 2009; Heath et al., 2009) and reduced tumor growth (Pozzi et al., 2013).

BHQ880, has been tested in patients with MM, in combination with approved anti-MBD therapy such zoledronic acid. BHQ880 may increase trabecular bone formation and block osteoclastogenesis and be beneficial in patients with relapsed or refractory MM (Iyer et al., 2014). A phase II clinical trial in evaluating BHQ880 use in MM patients that present with renal insufficiently has been recently completed although the results are yet to be published (NCT01337752).

Anti-sclerostin

A number of studies have demonstrated that inhibition of sclerostin may be exploited in disease characterized by high bone catabolic rate such as osteoporosis (McClung, 2017). Results from a Phase III trial showed that Romosozumab, a monoclonal antibody that binds and neutralises sclerostin, increased bone formation, decreased bone resorption and reduced the risk of vertebral fractures in postmenopausal women with osteoporosis (Cosman et al., 2016). Furthermore, Romosozumab was superior to the aldronate and teriparatide, in increasing bone formation and reducing bone resorption. Elevated serum levels of sclerostin have been found in MM patients, which correlated with disease stage, marker of bone remodelling and number of pathological fractures (Wang et al., 2014). Preclinical studies conducted in our laboratory, combining Scl-Ab with available anti-MM drugs, such as bortezomib and dexamethasone, showed that Romosozumab did not negatively affect their anti-MM activity thus promoting its use in combinational therapy to improve bone disease and inhibit tumor progression *in vitro* (Delgado-Calle et al., 2017). Others reported a superior effect of Romosozumab combined with zoledronic acid in increasing bone volume and resistance to fracture *in vivo* (McDonald et al., 2017). These studies suggest that targeting sclerostin improve bone disease, can be efficiently combined with anti-MM drugs and antiresorptive agents and may be of value in MBD. Recently, the FDA approved

Romosozumab for the treatment of osteoporosis in postmenopausal women with high fracture risk who are intolerant to other osteoporosis therapies. However, results from the ARCH study conducted by Amgen revealed that Romosozumab increased the risk of cardiovascular problems in patients compared to alendronate. These finding results in Amgen advising that Romosozumab should not be administered to patients who suffer myocardial infarction or stroke in the previous year.

Bruton's tyrosine kinase (BTK)

BTK is a non-receptor tyrosine kinase that plays a central role in the activation of downstream pathways associated with survival and proliferation of B cells (Petro & Khan, 2001). MM.1R dexamethasone resistant cells presented an upregulated BTK expression at both protein and mRNA levels, suggesting a possible role of BTK in the mechanism of dexamethasone resistance (Chauhan et al., 2002). Ibrutinib is a first-in-class, potent, once-daily, orally administered, covalently binding inhibitor of BTK. Selective inhibition of the BTK protein decreased MM-induced bone destruction by inhibiting osteoclastogenesis and suppressed tumor growth in an *in vivo* mouse model (Tai & Anderson, 2012). BTK inhibition reduced *in vivo* homing of MM cells to bone in the SCID-rab model, inhibited MM cell growth and survival and altered their adhesion to BMSCs (Bam et al., 2013). Very recently, a Phase II trial, involving relapsed and refractory multiple myeloma patients, showed that Ibrutinib, with or without weekly dexamethasone, demonstrated promising activity and was well tolerated (Richardson et al., 2018).

IL-6

IL-6 is a pro-inflammatory cytokine recognized as a key molecule in the development of MM (Al-Hujaily, Oldham, Hari & Medin, 2016). IL-6 is released by BM stem cells and binds to the IL-6 receptor (IL-6R) on myeloma cells stimulating their proliferation and survival (Burger, 2013) and promotes drug resistance . IL-6 is also produced by the MM cells, monocytes, endothelial cells, OBs and BM adipocytes and acts as a OC differentiation modulator, therefore is considered a cytokine that may be targetable for therapeutic purposes (Harmer, Falank & Reagan, 2018).

Siltuximab, a chimeric anti-IL-6 monoclonal antibody (mAb) (CNTO328) was tested in MM cell lines and in cells from refractory MM patients were it enhanced the cytotoxic effect of melphalan, dexamethasone, or bortezomib combined with dexamethasone (Hunsucker et al.,

2011). The phase I clinical trial had discouraging results, despite demonstrating the safety and tolerability of the drug, the efficacy of siltuximab was modest (Kurzrock et al., 2013). The phase II clinical trial, involving refractory or relapsed MM patients treated with siltuximab either alone or in combination with dexamethasone, reported an overall response rate of 17% in patients who received the combination therapy (Voorhees et al., 2013). Tocilizumab, a humanized anti-IL-6R mAb, is currently the subject of investigation in clinical cancer trials including MM (Matsuyama et al., 2011).

Preclinical studies

Transforming Growth Factor Beta

TGF-β has been implicated in a number of cancers that metastasize to bone. As a consequence of the increased bone resorption, TGF-β is released from the resorbed bone matrix and promotes MM cell growth. In bone, although the mechanism it still unclear, studies with genetically modified mice showed that TGF-β affects OB differentiation via a mechanism that involve SMAD3 binding to Runx2 promoter, preventing its transcriptional activity and also promotes osteoclastogenesis and bone resorption (reviewed in (Juarez & Guise, 2011)).

A number of recent studies have shown that a TGF-β inhibitor neutralising antibody (1D11) alone or in combination with conventional therapies such as bortezomib and zoledronic acid inhibit OC activity and increase OB differentiation and bone formation without affecting tumour burden (Nyman et al., 2016; Paton-Hough et al., 2019). In another study however, a TGF-β inhibitor, SRI31277 decreased MM tumour burden *in vivo* via a mechanism that involve Thrombospondin1 (TSP-1), a regulator of latent TGF-β activation (Lu et al., 2016).

A humanized anti- TGF-β antibody (GC1008) is currently in clinical trials in breast cancer (NCT01401062) (Formenti et al., 2018).

IL-17

Th17 T cells are T lymphocytes, capable of producing the pro-inflammatory cytokine IL-17 but not interferon-γ (IFN-γ) or IL-4, whose differentiation is mediated by TGF-β and IL-6 (Zhou et al., 2007). In MM patients' BM, the ratio between CD4+/CD25+ population components is imbalanced with Tregs almost being absent and Th17 highly increased

(Noonan, Marchionni, Anderson, Pardoll, Roodman & Borrello, 2010). Moreover, it has been demonstrated that IL-17 release promotes MM cell growth (Prabhala et al., 2010) and induces OC activation as well as Th17 cell number correlates with the degree of lytic bone lesions (Noonan, Marchionni, Anderson, Pardoll, Roodman & Borrello, 2010). A recent study showed that anti-IL-17A mAb (AIN457) significantly inhibited the growth of MM cell lines, as well as primary cells, and were able to overcome protective effects of BMSCs and significantly block *in vitro* OC differentiation (Prabhala et al., 2016). These data indicate Th17 as a potential drug target for the improvement of myeloma bone disease.

CD26

The OCs in the MM osteolytic lesions highly expresses CD26, a transmembrane glycoprotein with dipeptidyl peptidase IV (DPPIV) activity. A humanized anti-CD26 monoclonal antibody (HuCD26mAb) was able to impair human OC differentiation by modulating RANK-signalling pathway and inactivating downstream intracellular MKK3/6-p38MAPK pathway in OC precursor cells (Nishida et al., 2014). These results are encouraging foundation for using a bone-targeted therapy with anti-CD26 antibody in combination with systemic anti-MM therapy to reduce the occurrence of total skeletal related events.

miRNAs

Micro RNAs (miRNAs) are short non coding RNAs of about 18-24 nucleotides length, that target the 3'-untranslated region (3'-UTR) of mRNAs and inhibiting protein translation (Bartel, 2004). Recent studies showed that miRNA dysregulation increases progressively from healthy individuals to MGUS and MM patients (Pichiorri et al., 2008), indicating that miRNA modulation can be involved in the transition from MGUS to MM, with specific signatures for MGUS and MM being determined (Roccaro et al., 2009).

Single dysregulated miRNAs may be directly involved in critical functions for malignant plasma cells such as cell cycle control, DNA repair and interactions with cell components of the microenvironment. Therefore, it is considered that up-regulated miRNAs are driving survival and expansion of plasma cells (oncomiRs), while down-regulated miRNAs are inhibitory signals for proliferation and progression (tumor suppressor miRNAs, TS-miRs). The pleiotropic effects of miRNAs make them very suitable tools for designing novel therapeutic strategies. For instance, miRNA enforced expression (mimics or antagomiRs)

may be used against MM and its microenvironment (Rossi, Amodio, Di Martino, Caracciolo, Tagliaferri & Tassone, 2013). Several miRs are downregulated in MM (e.g. miR-15, -16, 34, -29b, -125b, 214, 137-197, -145 and -199). Their restoration by transfection of miR mimics within MM cells inhibited DNA synthesis, reduced proliferation and reduced the formation of tumoral driven new vasculature. Selective inhibition of the oncomiRs in MM (e.g. miR-21, -221/222, -125a) induced cell cycle arrest, impaired tumor cell proliferation and migration while promoting cell death (Rossi, Tagliaferri & Tassone, 2015). Recent preclinical studies have investigated ways to restore the physiological state of the BM microenvironment, independently from direct activity against the MM by modulating miRNAs that affect cell components of the BM microenvironment.

MiR-29b replacement within OCLs inhibited bone resorption activity, even in the presence of MM cells, by decreasing OCL responsiveness to RANKL stimulation and decreased pro osteolytic enzymes levels (Rossi et al., 2013). Pitari and coworkers described that BMSCs up-regulate miR-21 in the presence of MM cells contributing to a tumor favouring microenvironment (Pitari et al., 2015). Moreover, miR-21 is a critical oncomiR in MM and its expression is strictly related to BM IL-6 levels. MiR-21 contributes to the suppression of OPG, therefore miR-21 antagonistic strategies revert OPG suppression and restored RANKL/OPG to physiological levels. Treatment with miR-202 mimics restored its level within MM-related BMSCs, therefore overcame growth-promoting activity of the BMSC (Shen et al., 2016).

Concluding remarks

Despite the recent improvements in MM treatment, more than 80% of MM patients suffer from skeletal disease and its related complications, implying that new treatment strategies are needed. The excessive osteoclastic bone resorption and persistent decrease in osteoblastic bone formation that cause dramatic bone loss, severe bone pain and pathological fractures, markedly decrease the quality of life of MM patients. A better understanding of the cellular and molecular mechanisms that tightly regulate the interplay between bone marrow cells and MM cells, has led to the discovery and introduction of a number of encouraging therapeutic agents. For the last two decades BPs have been the mainstream of treatment for MBD, but since January 2018, denosumab was approved for patients with active MM with compromised renal function. However, the major challenge in the treatment of MBD is to repair bone damage that results in healing of lytic bone lesions. The inability of the conventional MM chemotherapies to affect OB activity and induce bone formation

demonstrate the need for the development of new safe and potent anabolic agents. Recent studies have shown that, proteasome inhibitors, in particular bortezomib, increase OB differentiation and may promote bone remineralization, independently from their anti-MM response. However, the extent, location, and patterns of bone mineralization vary among patients and are unpredictable.

Ongoing preclinical and clinical studies are evaluating the effect on bone remodelling of novel anti-MM agents, including proteasome inhibitor, ixazomib (NCT02499081), anti-CD38 antibody, daratumumab (NCT03475628) and Pim2 kinase inhibitors (NCT01456689) in patients with relapsed and /or refractory MM. The ultimate goal of the management of MBD is not only reducing tumor burden but also restoring balanced of bone remodelling in order to prevent further bone loss and potentially repair bone damage in MM patients.

Nomenclature of Targets and Ligands:

Key protein targets and ligands in this article are hyperlinked to corresponding entries in http://www.guidetopharmacology.org, the common portal for data from the IUPHAR/BPS Guide to PHARMACOLOGY (Harding et al., 2018), and are permanently archived in the Concise Guide to PHARMACOLOGY 2017/18 (Alexander et al., 2017).

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Conflicts of Interest

G.D.R. is a consultant to Amgen. S.M. and D.N.P. have no conflict of interests regarding the publication of this paper.

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Fig 1: MBD can cause excessive bone destruction accompanied by absent new bone formation in any bone. Radiographic images of osteolytic lesions in the fingers (left panel), skull (middle panel) and shoulder (right panel) of myeloma patients (Courtesy of Dr. Mankin, Massachusetts General Hospital).

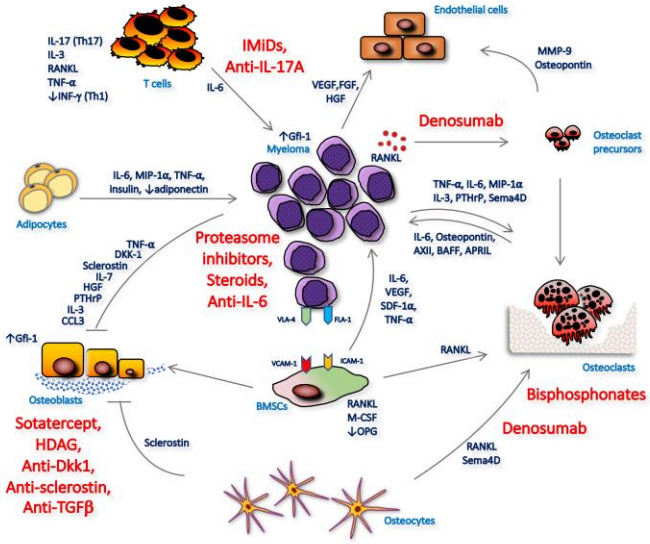

Fig 2: Cellular interactions and treatments of MBD. The excessive bone destruction and abnormal bone remodeling that characterizes MBD is the result of a complex interplay among the MM cells and the cells of the bone marrow microenvironment. MM cells stimulate osteoclast formation directly, through the production of several soluble factors (RANKL, MIP-1α, IL-3, and TNF-α, Sema4D and PTHrP) or by physical interaction with marrow stromal cells and osteocyte, increasing their production of osteoclastogenic factors. The newly formed osteoclasts secrete soluble factors (osteopontin, MIP-1α, IL-6, AXII, BAFF, and APRIL) that stimulate tumor growth. Moreover, matrixassociated growth factors (TGFβ, IGFs, FGF, PDGFs, and BMPs) are released from the resorbed bone and increase MM cells proliferation, exacerbating the osteolytic process. MM cells induce a profound suppression of bone formation by releasing osteoblast-inhibitory factors (DKK1, sclerostin, HGF, IL-7, and TNF-α) and inducing the release of sclerostin from osteocytes and TNF-α from marrow stromal cells. Immune cells, adipocytes and endothelial cells also enhance MBD due to increase secretion of factors that affects both MM cells and bone cells. The development of new potent anabolic agents in combination with both antiresorptive and anti-tumour agents appear to be the most promising strategies for healing MBD.